Cystic Fibrosis Foundation

November 20, 2000

Dockets Management Branch
(HFA-305)
Food and Drug Administration
5630 Fishers Lane, Room 1061
Rockville, MD 20862

RE: Docket Number: 00N-1409; Proposed Rule on Physical Medicine Devices; Revision of the Identification of the Iontophoresis Device

Dear Sir or Madam:

On behalf of patients with cystic fibrosis (CF), the Cystic Fibrosis Foundation (CFF) is concerned about the potential negative impact of the proposed rule to revise the identification of the iontophoresis device for the use of this device to diagnose patients with cystic fibrosis (CF).

The sweat test using the iontophoresis device has been the standard test to diagnose CF for more than 40 years. As a physician who has treated patients with CF for many years, I can assure you that sweat testing is essential for the diagnosis of these patients. Because of this, the CF Foundation routinely site visits all of its accredited centers and, during this visit, it documents that the center is in compliance with the NCCLS guidelines for sweat testing. For more information, we have enclosed a recent memorandum to the CFF Care Centers from September 21, 2000 and the consensus document entitled "Sweat Testing: Sample Collection and Quantitative Analysis; Approved Guideline- Second Edition" from NCCLS (C34-A2, (ISBN 1-56238-407-4)).

We believe that the requirement in the proposed rule for labeling of the pilocarpine for use with the iontophoresis device could inadvertently halt the use of this test for CF diagnostic purposes. Since the makers of this device have no control over the plans of the manufacturers of pilocarpine to obtain labeling, and this device and drug combination is essential for the sweat test to diagnose CF, this rule could undermine the ability of our physicians to properly diagnose CF via an accurate method, which is free from adverse reactions.

It is not clear from the information in the Federal Register (August 22, 2000, Volume 65, Number 163, page 50949) whether or not the proposed rule impacts the use of the iontophoresis device for diagnosis of cystic fibrosis or if it only impacts non-CF uses. In outlining the existing rule, the notice states that "the regulation defines a class II device as a device intended for use in the diagnosis of cystic fibrosis or for other uses, if the labeling of the drug intended for use with the device bears adequate directions for the

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National Office
6931 Arlington Road Bethesda, Maryland 20814
(301) 951-4422 (800) FIGHT CF (800) 344-4823 Fax: (301) 951-6378 Internet: www.cff.org E-mail: info@cff.org

device's use with that drug.... A class III iontophoresis device is intended for uses other than those specified for the class II device."

The proposed revision states that FDA is "revoking the class III identification. Any device that is not substantially equivalent to the class II device would be considered a postamendment device that is automatically classified in class III..." This could be interpreted to state that the proposed rule does not affect the classification of the device as a class II device for use in diagnosing patients with CF. Alternatively, it could require that, in order to properly be classified as a class II device, further labeling of the associated drug products for CF diagnostic purposes would be necessary.

If the proposed rule is intended to impact the use of the device in diagnosing CF, we respectfully request that you revisit this issue. We believe it would be best to continue classifying the iontophoresis device as a Class II device for purposes of CF diagnosis to enable continued use of the device.

The CF Foundation hopes to play a proactive role in proper classification of the iontophoresis device for use in diagnosing CF, because the impact of new regulations on this classification could make it more difficult to identify manufacturers to make this device for CF diagnostic testing. If the bar is raised further, the use of this device in diagnosing CF with the sweat test may no longer be feasible.

Therefore, we respectfully request clarification on the impact of this proposed rule on the use of iontophoresis devices for diagnosing CF with the sweat test. We want to work with you to identify the best means to appropriately classify and label this device to ensure the use of this device for diagnosing patients with CF. It would be a tremendous disservice to patients and families if the sweat test were no longer available.

Please contact us at 301-951-4422 to identify ways in which we can work together to address this issue.

Sincerely,

Preston W. Campbell, III, M.D.
Executive Vice President for Medical Affairs

Cystic Fibrosis Foundation

DATE: September 21, 2000

MEMO TO: CF Center Directors
CF Affiliate Directors
CF Adult Program Directors

FROM: CFF Center Committee

SUBJECT: Sweat Testing Guidelines

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Enclosed please find the final version of the NCCLS document: SWEAT TESTING: SAMPLE COLLECTION AND QUANTITATIVE ANALYSIS: APPROVED GUIDELINE: C34-A. This will replace the previous draft you received in 1996. Although this document is a guideline, it should become the basis for your sweat testing procedures. We want to point out that there are several criteria that the CFF requires. **Failure to use the appropriate techniques outlined below will require immediate corrective action or a Center will not be accredited:**

1. The laboratory must perform the quantitative pilocarpine iontophoresis sweat test according to the procedures outlined in NCCLS document C34-A.
2. The iontophoresis equipment must be battery powered and regularly inspected.
3. The minimum age for testing is 48 hours.
4. Only the arms and legs are used as collection sites. The iontophoresis current does not cross the heart.
5. Sweat must be collected on gauze or filter paper or in a Wescor Macroduct coil following iontophoresis using USP grade pilocarpine.
6. Sweat must be collected for **no more than** 30 minutes.
7. The minimum acceptable sample for analysis from a single site using 2 x 2 inch gauze or filter paper for stimulation and collection is 75 mg collected in 30 minutes. Using the Wescor Macroduct coil system, the minimum acceptable sample is 15 uL collected in 30 minutes. These requirements are based on the stimulation and collection parameters described in the NCCLS document. (Note that previous CFF documents had required 50 mg for a minimum sample volume.)
8. The incidence of insufficient samples must be investigated and resolved if it exceeds 5%.
9. It is recommended that the collection and analysis be performed in duplicate.
10. Insufficient samples must not be pooled for analysis.
11. Collection and analytical procedures must be designed to minimize evaporation and/or contamination. For specific techniques, refer to NCCLS document C34-A, Section 8.1.3.1 and 8.1.4.
12. Sweat must be quantitatively analyzed for chloride, with or without sodium, by one of the following methods:
    a. Chloride by coulometric titration using a chloridometer
    b. Chloride by a manual titration using the Schales and Schales mercuric nitrate procedure

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c. Sodium by flame photometry
d. Chloride or sodium by automated analyzers employing ion-selective electrodes which have been systematically validated against the methods described in a-c above*

It is not appropriate to perform the sweat test using:

a. Direct application of a chloride electrode to the patient's skin
b. Chloride precipitation reaction employing a patch placed directly on the patient's skin
c. Measuring only potassium
d. Osmolality
e. Conductivity
f. Any other screening (non-quantitative) tests

13. Perform and evaluate quality control with every sweat analysis run using two levels of controls per the clinical Laboratory Improvement Act of 1988 (CLIA '88)
14. It is recommended that the sweat test be included in the laboratory's overall evaluation of CQI (Continuous Quality Improvement)
15. Sweat samples must be appropriately labeled for patient identification throughout sweat collection and analysis. Reagents must be appropriately labeled.
16. Appropriate reference values for sweat chloride must be used: <40 mmol/L = negative; 40-60 mmol/L = borderline/indeterminate; > 60 mmol/L = consistent with the diagnosis of CF. NOTE: Sweat chloride values less than 40 mmol/L have been documented in genetically proven CF patients. Clinical correlation is necessary.
17. The reportable range of results for sweat chloride is 1-165 mmol/L.
18. All laboratories must document successful performance in the College of American Pathologists (CAP) proficiency testing survey for sweat test analysis.
19. We strongly recommend that the Center Director review all sweat test results.
20. All positive tests must be repeated at a different time.
21. Sweat testing must be available at least 3 days/week.
22. Sweat testing must e performed on a sufficient number of patients by a limited number of experienced, well-trained personnel who pass periodic documented competency testing. CLIA '88 and JCAHO require that new employees demonstrate competency every 6 months for the first year and annually thereafter.
23. The laboratory must have a copy of the above referenced NCCLS Guidelines document C34-A.

We hope this information will be helpful to you and your laboratory director. Thank you for your assistance in maintaining the CF Center network as a model of excellence.

* There is a concern for the sensitivity of these analyzers in the lower electrolyte concentrations. (Automated analyzers using ion-selective electrodes for sweat chloride and/or sodium are different from the *in situ* or direct reading chloride electrode which is applied to the patient's skin).

C34-A2
Vol. 20 No. 14

Replaces C34-A
Vol. 13 No. 2

Sweat Testing: Sample Collection and Quantitative Analysis; Approved Guideline—Second Edition

This document addresses appropriate methods of collection and analysis, quality control, and the evaluation and reporting of test results.

A guideline for global application developed through the NCCLS consensus process.

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NCCLS is an international, interdisciplinary, nonprofit, standards-developing, and educational organization that promotes the development and use of voluntary consensus standards and guidelines within the healthcare community. It is recognized worldwide for the application of its unique consensus process in the development of standards and guidelines for patient testing and related healthcare issues. NCCLS is based on the principle that consensus is an effective and cost-effective way to improve patient testing and healthcare services.

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An NCCLS document is published as a standard, guideline, or committee report.

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CONSENSUS PROCESS

The NCCLS voluntary consensus process is a protocol establishing formal criteria for:

- the authorization of a project
- the development and open review of documents
- the revision of documents in response to comments by users
- the acceptance of a document as a consensus standard or guideline.

Most NCCLS documents are subject to two levels of consensus—"proposed" and "approved." Depending on the need for field evaluation or data collection, documents may also be made available for review at an intermediate (i.e., "tentative") consensus level.

Proposed An NCCLS consensus document undergoes the first stage of review by the healthcare community as a proposed standard or guideline. The document should receive a wide and thorough technical review, including an overall review of its scope, approach, and utility, and a line-by-line review of its technical and editorial content.

Tentative A tentative standard or guideline is made available for review and comment only when a recommended method has a well-defined need for a field evaluation or when a recommended protocol requires that specific data be collected. It should be reviewed to ensure its utility.

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NCCLS standards and guidelines represent a consensus opinion on good practices and reflect the substantial agreement by materially affected, competent, and interested parties obtained by following NCCLS's established consensus procedures. Provisions in NCCLS standards and guidelines may be more or less stringent than applicable regulations. Consequently, conformance to this voluntary consensus document does not relieve the user of responsibility for compliance with applicable regulations.

COMMENTS

The comments of users are essential to the consensus process. Anyone may submit a comment, and all comments are addressed, according to the consensus process, by the NCCLS committee that wrote the document. All comments, including those that result in a change to the document when published at the next consensus level and those that do not result in a change, are responded to by the committee in an appendix to the document. Readers are strongly encouraged to comment in any form and at any time on any NCCLS document. Address comments to the NCCLS Executive Offices, 940 West Valley Road, Suite 1400, Wayne, PA 19087, USA.

VOLUNTEER PARTICIPATION

Healthcare professionals in all specialties are urged to volunteer for participation in NCCLS projects. Please contact the NCCLS Executive Offices for additional information on committee participation.

Sweat Testing: Sample Collection and Quantitative Analysis; Approved Guideline—Second Edition

Abstract

NCCLS document C34-A2—*Sweat Testing: Sample Collection and Quantitative Analysis; Approved Guideline—Second Edition* is a guideline for the performance of the sweat test for the diagnosis of cystic fibrosis. The primary audience includes laboratory and clinical personnel responsible for collecting, analyzing, reporting, and evaluating sweat test results. Sweat stimulation, collection, and the quantitative measurement of sweat chloride and sodium are described, with an emphasis on avoiding evaporation and contamination. Quality control issues associated with sweat testing are discussed, along with analytical and biological sources of error.

NCCLS. *Sweat Testing: Sample Collection and Quantitative Analysis; Approved Guideline—Second Edition.* NCCLS document C34-A2 (ISBN 1-56238-407-4). NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2000.

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Vicky A. LeGrys, Dr.A., M.T.(ASCP)
Beryl J. Rosenstein, M.D.
Basil T. Doumas, Ph.D.
W. Gregory Miller, Ph.D.
Paul D'Orazio, Ph.D.
John H. Eckfeldt, M.D., Ph.D.
Susan A. Evans, Ph.D.
Gary A. Graham, Ph.D., DABCC
Gary L. Myers, Ph.D.
Patrick J. Parsons, Ph.D.
Noel V. Stanton, M.S.

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Committee Membership

Area Committee on Clinical Chemistry and Toxicology

Basil T. Doumas, Ph.D. **Chairholder**	**Medical College of Wisconsin** **Milwaukee, Wisconsin**
W. Gregory Miller, Ph.D. **Vice-Chairholder**	**Virginia Commonwealth University** **Richmond, Virginia**
Paul D'Orazio, Ph.D.	Instrumentation Laboratory Lexington, Massachusetts
John H. Eckfeldt, M.D., Ph.D.	Fairview-University Medical Center Minneapolis, Minnesota
Susan A. Evans, Ph.D.	Dade Behring Inc. Deerfield, Illinois
Gary A. Graham, Ph.D., DABCC	Ortho-Clinical Diagnostics Rochester, New York
Gary L. Myers, Ph.D.	Centers for Disease Control and Prevention Atlanta, Georgia
Patrick J. Parsons, Ph.D.	New York State Department of Health Albany, New York
Noel V. Stanton, M.S.	University of Wisconsin Madison, Wisconsin
Advisors	
Judith T. Barr, Sc.D.	Northeastern University Boston, Massachusetts
Stanley Bauer, M.D.	Beth Israel Medical Center New York, New York
George N. Bowers, Jr., M.D.	Hartford Hospital Hartford, Connecticut
Robert W. Burnett, Ph.D.	Hartford Hospital Hartford, Connecticut
Mary F. Burritt, Ph.D.	Mayo Clinic Rochester, Minnesota
Kevin D. Fallon, Ph.D.	Instrumentation Laboratory Lexington, Massachusetts

Advisors (Continued)

Carl C. Garber, Ph.D.	Quest Diagnostics, Incorporated Teterboro, New Jersey
Harvey W. Kaufman, M.D.	Quest Diagnostics, Incorporated Teterboro, New Jersey
Jan S. Krouwer, Ph.D.	Bayer Diagnostics Medfield, Massachusetts
Victoria M. Leitz, Ph.D.	International Biomedical Consultants Hilton Head, South Carolina
Richard R. Miller, Jr.	Dade Behring Inc. Newark, Delaware
Robert F. Moran, Ph.D., FCCM, FAIC	mvi Sciences Methuen, Massachusetts
Richard B. Passey, Ph.D.	University of Oklahoma Oklahoma City, Oklahoma
Edward A. Sasse, Ph.D.	Medical College of Wisconsin Milwaukee, Wisconsin
Richard S. Schifreen, Ph.D.	Promega Corporation Madison, Wisconsin
Bette Seamonds, Ph.D.	National Academy of Clinical Biochemistry Swarthmore, Pennsylvania
Beth Ann Wise, M.T.(ASCP), M.S.Ed. *Staff Liaison*	NCCLS Wayne, Pennsylvania
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St. Barnabas Medical Center (NJ)
St. Boniface General Hospital (Winnipeg, Canada)
St. Elizabeth Hospital (NJ)
St. John Hospital and Medical Center (MI)
St. John Regional Hospital (St. John, NB, Canada)
St. Joseph Medical Center (MD)
St. Joseph Hospital (NE)
St. Joseph Mercy – Oakland (MI)
St. Joseph's Hospital - Marshfield Clinic (WI)
St. Luke's Hospital (PA)
St. Luke's Regional Medical Center (IA)
St. Mary Medical Center (IN)
St. Mary of the Plains Hospital (TX)
Salina Regional Health Center (KS)
San Francisco General Hospital (CA)
Santa Cabrini Hospital (Montreal, PQ Canada)
Santa Clara Valley Medical Center (CA)
Seoul Nat'l University Hospital (Korea)
Shanghai Center for the Clinical Laboratory (China)
Shands Healthcare (FL)
SmithKline Beecham Clinical Laboratories (GA)
SmithKline Beecham Clinical Laboratories (WA)
South Bend Medical Foundation (IN)
Southern California Permanente Medical Group
South Western Area Pathology Service (Australia)
Speare Memorial Hospital (NH)
Speciality Laboratories, Inc. (CA)
Stanford Health Services (CA)
State of Washington Department of Health
Stormont-Vail Regional Medical Center (KS)
Sun Health-Boswell Hospital (AZ)
Sunrise Hospital and Medical Center (NV)
Sutter Health (CA)
Tampa General Hospital (FL)
Tripler Army Medical Center (HI)
Tulane Medical Center Hospital & Clinic (LA)
UCSF Medical Center (CA)
UNC Hospitals (NC)
Unilab Clinical Laboratories (CA)
University of Alberta Hospitals (Canada)
University of Chicago Hospitals (IL)
University of Florida
University Hospital (IN)
University Hospital (Gent) (Belgium)
University Hospital (London, Ontario, Canada)
The University Hospitals (OK)
University of Massachusetts Lowell
University of Medicine & Dentistry, NJ University Hospital
University of Michigan
University of the Ryukyus (Japan)
University of Virginia Medical Center .
University of Washington
UPMC Bedford Memorial (PA)
USAF Medical Center (OH)
UZ-KUL Medical Center (Belgium)
VA (Dayton) Medical Center (OH)
VA (Denver) Medical Center (CO)
VA (Martinez) Medical Center (CA)
VA (San Diego) Medical Center (CA)
VA (Tuskegee) Medical Center (AL)
VA Outpatient Clinic (OH)
Vejle Hospital (Denmark)
Via Christi Regional Medical Center (KS)
Virginia Department of Health
Viridae Clinical Sciences, Inc. (Vancouver, BC, Canada)
Walter Reed Army Institute of Research (MD)
Warde Medical Laboratory (MI)
Warren Hospital (NJ)
Washoe Medical Center (NV)
Watson Clinic (FL)
Wilford Hall Medical Center (TX)
William Beaumont Hospital (MI)
Williamsburg Community Hospital (VA)
Wilson Memorial Hospital (NY)
Winchester Hospital (MA)
Winn Army Community Hospital (GA)
Wishard Memorial Hospital (IN)
Womack Army Medical Center (NC)
Yan Chai Hospital (P.R. China)
Yonsei University College of Medicine (Korea)
York Hospital (PA)
Zale Lipshy University Hospital (TX)

Contents (Continued)

Foreword

The quantitative measurement of chloride in sweat (commonly called the "sweat test") is used to confirm the diagnosis of cystic fibrosis (CF). With an approximate incidence of 1:3,200 in the United States, CF is the most common lethal genetic disease within the Caucasian population. It is an autosomal recessive disorder characterized by viscous secretions that affect the exocrine glands, primarily in the lungs and pancreas. Patients with CF have an increased concentration of sodium, chloride, and potassium in their sweat. The criteria for the diagnosis of CF include: the presence of one or more characteristic phenotypic features, or a history of CF in a sibling, or a positive newborn screening test result; and an increased sweat chloride concentration by pilocarpine iontophoresis on two or more occasions, or identification of two CF mutations or demonstration of abnormal nasal epithelial ion transport.[1]

The sweat test has been reported to have unacceptably high false-positive (up to 15%) and false-negative (up to 12%) rates attributable to inaccurate methodology, technical error, and patient physiology.[2-7] Comprehensive guidelines addressing the collection of sweat and the quantitative measurement of electrolytes in sweat are needed. Improvement in the performance of such tests can only occur when laboratory scientists and clinicians are aware of appropriate methods of collection and analysis, quality control, and evaluation of results. This document describes, in detail, the quantitative pilocarpine iontophoresis test for the determination of sweat chloride, including techniques to minimize the potential for false-positive and false-negative test results. Because some laboratories analyze sweat sodium in addition to chloride, a procedure for sodium is found in Appendix F. Nonselective screening methods, such as conductivity and osmolality, are also mentioned; such methods measure other analytes in addition to chloride. Other methods for measuring sweat electrolytes after pilocarpine iontophoresis exist but are not included in the guideline. Some of these methods are documented as having significant analytical problems.[2-7]

The Cystic Fibrosis Foundation requires that, at accredited Cystic Fibrosis Care Centers, sweating be stimulated by pilocarpine iontophoresis and collected in either gauze or filter paper, or microbore tubing followed by quantitative measurement of chloride. At alternative sites, as a screening procedure, conductivity may be measured (see Section 8.3.1). Patients with a sweat conductivity value of 50 mmol/L (equivalent NaCl) or above should have a quantitative measurement of sweat chloride.[8]

Key Words

Chloridometer, iontophoresis, sweat chloride, sweat testing

Sweat Testing: Sample Collection and Quantitative Analysis; Approved Guideline—Second Edition

1 Introduction

Because the sweat test has been reported to have unacceptably high false-positive and false-negative rates attributable to inaccurate methodology, technical error, and patient physiology,[2-7] comprehensive guidelines addressing the collection of sweat and the quantitative measurement of electrolytes in sweat are needed. Improvement in the performance of such tests can only occur when laboratory scientists and clinicians are aware of appropriate methods of collection and analysis, quality control, and evaluation of results. This document describes, in detail, the quantitative pilocarpine iontophoresis test for the determination of sweat chloride, including techniques to minimize the potential for false-positive and false-negative test results.

2 Scope

The following procedures are described: the stimulation and collection of sweat and the quantitative measurement of chloride; sweat stimulation by pilocarpine iontophoresis (specific precautions are noted); and sweat collection in filter paper, gauze, and microbore tubing, with emphasis on avoiding evaporation and contamination. Sweat chloride determination is described using coulometric titration. Nonselective screening methods, such as conductivity and osmolality, are mentioned. While chloride determinations provide greater discrimination in CF, sweat sodium determination by flame photometry is measured in some European countries, and a procedure is included in Appendix F. Quality control issues are discussed, along with analytical and biological sources of error. This document is primarily directed towards laboratory and clinical personnel responsible for collecting, analyzing, reporting, and evaluating sweat chloride test results.

3 Definitions [a]

Calibrator, *n* - **1)** A material or device of known, or assigned quantitative characteristics (e.g., concentration, activity, intensity, reactivity, responsiveness) used to adjust the output of a measurement procedure or to compare the response obtained with the response of a test specimen and/or sample. **NOTES:** a) The quantities of the analytes of interest in the calibration material are known within limits ascertained during its preparation and may be used to establish the relationship of an analytical method's response to the characteristic measured for all methods or restricted to some; b) Calibration materials with different amounts of analytes may be used to establish a calibration or response "curve" over a range of interest.

Control, *n* – A device, solution, or lyophilized preparation intended for use in the quality control process. **NOTES:** a) The expected reaction or concentration of analytes of interest are known within limits ascertained during preparation and confirmed in use; b) Control materials are generally not used for calibration in the same process in which they are used as controls.

Iontophoresis, *n* - The migration of small ions in an electrical field; **NOTE:** In the sweat test, pilocarpine is iontophoresed into the skin to stimulate sweating.

[a] Some of these NCCLS definitions are found in NCCLS document NRSCL8—*Terminology and Definitions for Use in NCCLS Documents*. For more detailed source information, please refer to the most current edition of that document.

Quality assurance, *n* - All the planned and systematic activities implemented within the quality system and demonstrated as needed, to provide adequate confidence that an entity will fulfill requirements for quality.

Quality control, *n* - The operational techniques and activities that are used to fulfill requirements for quality.

Steatorrhea, *n* - The presence of excessive fecal fat.

4 Precautions

4.1 Standard Precautions

Because it is often impossible to know what might be infectious, all human blood specimens are to be treated as infectious and handled according to "standard precautions." Standard precautions are new guidelines that combine the major features of "universal precautions and body substance isolation" practices. Standard precautions cover the transmission of any pathogen and thus are more comprehensive than universal precautions which are intended to apply only to transmission of blood-borne pathogens. Standard precaution and universal precaution guidelines are available from the U.S. Centers for Disease Control and Prevention (*Guideline for Isolation Precautions in Hospitals.* Infection Control and Hospital Epidemiology. CDC. 1996;Vol 17;1:53-80.), [MMWR 1987;36(suppl 2S):2S-18S] and (MMWR 1988;37:377-382, 387-388). For specific precautions for preventing the laboratory transmission of blood-borne infection from laboratory instruments and materials; and recommendations for the management of blood-borne exposure, refer to NCCLS document M29—*Protection of Laboratory Workers from Instrument Biohazards and Infectious Disease Transmitted by Blood, Body Fluids, and Tissue.*

4.2 Procedural Precautions

Use powder-free gloves when collecting and analyzing sweat, because powder can affect the weight measurements and contaminate the samples.

4.3 Chemical Hygiene

Prepare all solutions requiring the use of concentrated acids and bases in accordance with all applicable federal guidelines. In addition, laboratory personnel should refer to the laboratory's chemical hygiene plan.

4.4 Verification of Analytical Methods

Follow the applicable state and federal guidelines for verification of analytical methods and instruments.

4.5 Burns

Burns to the patient's skin after iontophoresis are extremely rare. The burns can occur at either electrode and range from tiny, black dots the size of pinholes to larger, crater-like lesions. If a burn does occur, rinse the affected area well with distilled or deionized water, and, if severe, seek appropriate medical attention. Do not collect sweat over the area of the burn. To minimize the possibility of a burn, observe the following steps:

4.5.1 Cleanse the Skin

Clean the skin with distilled or deionized water before attaching the electrodes. This removes dead surface skin cells and hydrates the top skin layer. It also removes any contaminating lotions or creams that might interfere with the iontophoresis.

4.5.2 Maintain a Wet Interface

Maintain a wet interface with the skin during iontophoresis.

(1) If using iontophoresis reagents in gel form:

- Avoid prolonged exposure of the gel to the air. If the gel surface appears to have lost some of its wetness, apply a drop of distilled or deionized water to the skin or to the gel just before attaching the gel-fitted electrode to the extremity.

- As an added precaution, leave the skin damp after washing and before iontophoresis.

(2) If using reagents on gauze or filter paper:

- The gauze or filter paper must be thoroughly saturated with reagents during the iontophoresis.

4.5.3 Limit Iontophoretic Current

Burn potential increases with the magnitude and duration of iontophoretic current. At the beginning of the iontophoresis, set the rheostat for maximum resistance [lowest possible current about 0.5 milliamperes (mA)], then bring the current up slowly to a maximum of 4 mA and maintain it there for five minutes. During the five minutes, monitor the current closely to ensure that it does not exceed 4 mA.

4.5.4 Avoid Skin-Electrode Contact

(1) If using iontophoresis reagents in gel form:

Inspect gel discs for cracks or any structural defect that would allow direct skin-electrode contact. Discs exposed to air for a long time will lose water and shrink in size. Reject any disc with a diameter that is significantly smaller than that of the electrode. Do not use any disc that is internally fractured or that is crumbled as a result of freezing. The disc should fit snugly into the recess of the plastic electrode assembly.

(2) If using reagents on gauze or filter paper:

To prevent the possibility of direct electrode contact with the skin, which will cause a burn, the gauze or filter paper should be slightly larger than the bare electrodes. Position the electrodes carefully on the gauze or filter paper so that a generous margin of the gauze or filter paper extends from under each edge of the electrode.

4.5.5 Attach Electrodes Firmly

Always apply the electrodes using firm tension in the attachment straps to ensure that a uniform, low-resistance interface exists between the electrolyte reservoir and the skin. This minimizes patient discomfort and reduces the possibility of burn formation.

4.5.6 Keep the Electrode Surfaces Clean

It is important to keep the electrode surfaces clean and free of any surface oxidation that might increase electrical resistance between the electrode and the electrolyte reservoir. Emery cloth can be used to clean the electrodes. Do not use commercial abrasive metal cleaners because of the potential for introduction of toxic chemicals into the skin during iontophoresis.

4.6 Electrical Malfunctions

To minimize the risk to the patient, follow these guidelines:

- Use a battery-powered iontophoresis system. This avoids the possibility of the patient being subjected to line voltage in the event of a component failure.

- It is desirable for the iontophoresis system to have a transistor current-limiting circuit that limits the amount of current delivered.

- The iontophoresis system should undergo a documented, regular maintenance procedure by medical engineering personnel for voltage leak and current control.

- Iontophoresis should not be performed on a patient receiving oxygen by an open delivery system. While the possibility of an explosion due to the generation of an electrical spark is remote, it cannot be ignored. Often these patients can temporarily receive oxygen via a face mask or nasal cannula, in which case sweat testing can be done.

4.7 Allergic Reactions

Allergic reactions to pilocarpine iontophoresis are extremely rare.[9] If, however, after the iontophoresis and/or collection, an area of diffuse inflammation and urticaria (hives) occurs, immediately discontinue the procedure and do not assay the sweat.

Keep an anaphylaxis treatment kit in the area for use by trained personnel or ensure that an alternate plan for treatment in case of emergencies is in place. Store the kit at room temperature, and make sure it includes the following items:

- 1-mg/mL epinephrine ampule(s),
- 1-mL tuberculin syringe(s),
- 50-mg diphenhydramine hydrochloride capsule(s),
- 25-mg diphenhydramine hydrochloride elixir in 10-mL aliquots, and
- alcohol preparation pad(s).

5 Principle

The procedure involves:

- sweat stimulation,
- sweat collection, and
- quantitative sweat measurement for chloride.

Common clinical indications for measuring chloride in sweat include a positive family history for cystic fibrosis; neonatal intestinal obstruction; steatorrhea; rectal prolapse; chronic cough; chronic wheezing; persistent or recurrent respiratory tract infections; nasal polyps; and failure to thrive. However, it is

important to consider the diagnosis of CF and perform such testing in patients with a wide variety of signs and symptoms (see Appendix A).

5.1 Sweat Stimulation

Localized sweating can be produced by the iontophoresis of the cholinergic drug pilocarpine nitrate into an area of skin. Iontophoresis uses an electric voltage so that an ionized drug carries current into the skin. The positively charged pilocarpine ions move away from the positive electrode and into the skin where they stimulate the sweat glands. A negative electrode with a dilute electrolyte solution is applied to the same limb.

5.2 Sweat Collection

After stimulation (Section 8.1), the skin is cleansed with distilled or deionized water and thoroughly dried. Sweat can then be collected by two different methods.

5.2.1 Gauze or Filter Paper

Using absorbent pad collection, the preweighed gauze or filter paper is placed over the site of the positive electrode and covered with a paraffin wax film to prevent evaporation. After collection (Section 8.1), the gauze or filter paper is removed and reweighed. The increase in the weight corresponds to the volume (mass/density) of the sweat collected. The relative density of sweat ranges from 1.001 to 1.008.[10] The sweat is then separated from the gauze or filter paper via elution (Section 8.2.2 and Appendix F, Section F2.2), and an aliquot is used for chloride determination.[11]

5.2.2 Microbore Tubing

Sweat can also be collected into self-harvesting, transparent, flexible, microbore tubing (Section 8.1.4). The collector consists of a slightly concave disc. A hole in the center of the disc admits a small, plastic microbore tube that passes through the disc and is coiled on the upper side of the disc. Sweat is forced through the central hole by hydrostatic pressure and is collected in the microbore tubing. A small amount of blue dye is painted on the bottom of the collecting disc, which allows for visualization of the collected sweat. After adequate specimen collection (Section 8.1.5), the tube is cut free from the disc for the measurement of chloride.[12,13]

5.2.3 Collection Time

The collection time for sweat is generally 30 minutes. Extension of this time can result in a sample taken from less than maximally stimulated glands and can lead to a false-negative result.[11,14]

5.3 Measurement of Chloride in Sweat

5.3.1 Units of Measurement

This guideline uses Système International d'Unités (SI) and provides measurements in millimole per liter (mmol/L). Results can be converted to milliequivalent per liter (mEq/L) by multiplying by 1.0, because the valence of chloride is 1.

5.3.2 Recommended Methods

After the collection of an adequate amount of sweat, it can be analyzed for chloride using coulometric titration with a chloridometer.

5.3.3 Other Methods

Analytical instrumentation and methodology for sweat determination should be approved by appropriate regulatory requirements (i.e., in the U.S., the Food and Drug Administration) for use on sweat specimens, or the methodology must be internally validated (see NCCLS documents on Evaluation Protocols listed in "Related NCCLS Publications"). Most analytical systems that employ ion-selective electrode (ISE) measurements of chloride in serum and other body fluids have not been systematically validated for sweat electrolyte determination. If such systems are employed, the user must validate the methods against traditional quantitative procedures described in the document. There is a concern when using ISEs to measure sweat chloride and/or sodium that the sensitivity at the lower concentrations could compromise the accuracy and precision of the results.

Other manual chloride methods, such as Schales and Schales, can be used if accuracy and precision can be validated.[15]

6 Apparatus and Equipment

6.1 Iontophoresis Equipment

6.1.1 Current Source

If the current source is manually controlled, a milliameter should be supplied that enables the operator to keep the current below 4 mA. For safety reasons, the current source should be battery-powered. Instruments designed with a voltage of 22.5 V are sufficient for iontophoresis involving sweat collection onto gauze or filter paper. Somewhat more voltage is used in automatically controlled units using smaller electrodes. On automatically controlled units, a low-battery indicator is helpful. (Refer to Appendix B for a discussion of current density.)

6.1.2 Electrodes

The electrodes are made of copper or stainless steel. The gauze or filter paper containing the pilocarpine solution and the electrolyte solution must be larger than the electrode. For example, an electrode that is 3.8 x 3.8 cm (1.5 x 1.5 in) would use a 5.1 x 5.1-cm (2.0 x 2.0-in) gauze square. If the electrodes are larger than 2.5 x 2.5 cm (1.0 x 1.0 in), they should be sufficiently pliable to be bent to fit the curvature of the patient's forearm or leg. Electrodes designed to be used with the gel discs should provide a snug fit for the gel disc into the electrode assembly. The electrodes should have central posts or some other device that allows the attachment of straps. The electrode surface should be smooth and free of irregularities.

6.1.3 Electrode Straps

Use suitable straps (e.g., rubber or hook-in-loop fabric) to secure the electrodes on the arm or leg.

6.1.4 Analytical Balance

An analytical balance is used if the sweat is collected on gauze or filter paper. The balance should be sensitive to 0.0001 g.

6.1.5 Scissors

6.1.6 Nippers

If the sweat is collected in microbore tubing, nippers are used. They are supplied by the manufacturer of the collection system.

6.1.7 Sweat Dispenser or Tuberculin Syringe

If the sweat is collected in microbore tubing, sweat dispensers or tuberculin syringes are used. The sweat dispenser is supplied by the manufacturer of the collection system.

6.2 Chloridometer

Use a chloridometer to determine sweat chloride concentration.

7 Materials and Reagents

7.1 Iontophoresis Materials

7.1.1 Water

Distilled or deionized water should be used. Refer to NCCLS document C3—*Preparation and Testing of Reagent Water in the Clinical Laboratory*, for water specifications.

7.1.2 Pilocarpine Nitrate Solution

A 7.4- to 18.4-mmol/L (2- to 5-g/L; 0.2 to 0.5 % w/v) solution of pilocarpine nitrate is used (see Appendix C). The source of the pilocarpine nitrate should be USP grade or equivalent. Many laboratories use a 14.7-mmol/L (4-g/L; 0.4% w/v) solution of pilocarpine. This is prepared by dissolving 1.2 g of pilocarpine nitrate in 300 mL of distilled or deionized water. This solution is stable for three months at 4 °C and one month at room temperature.

The pilocarpine nitrate is also available in an 18.4-mmol/L (5-g/L; 0.5% w/v)-concentration gel disc. The gel discs are stored at 4 °C and are stable until the expiration date indicated by the manufacturer. Do not freeze.

7.1.3 Electrolyte Solution

A dilute electrolyte solution is required for the negative electrode (cathode). In order to negate the possibility of contamination of the sweat sample with chloride from an electrolyte solution, 0.05-mol/L magnesium sulfate ($MgSO_4$) or 0.01-mol/L sulfuric acid (H_2SO_4) solution is recommended.

A 0.05-mol/L $MgSO_4$ solution is prepared by dissolving 12.4 g $MgSO_4 \cdot 7H_2O$ in a sufficient volume of distilled or deionized water to bring the total volume to 1 L. The solution is stable for six months at room temperature.

A 0.01-mol/L H_2SO_4 solution is prepared by adding 0.56 mL of concentrated sulfuric acid to 500 mL of distilled or deionized water in a liter volumetric flask and diluting to 1 L with distilled or deionized water. This is stable for six months at room temperature.

Alternatively, a second pilocarpine nitrate gel disc can be used at the negative electrode.

7.1.4 Gauze Pads and Tissues

Use 10.2 x 10.2-cm (4 x 4-in) gauze pads or laboratory tissues to clean and dry the patient's skin.

7.1.5 Gauze and Filter Paper

Use gauze squares or filter paper as the absorbent pad for the iontophoresis reagents. These should be slightly larger than the electrodes but smaller than the patient's extremity to ensure contact.

7.1.6 Emery Cloth

Emery cloth should be available for cleaning the electrodes.

7.1.7 Anaphylaxis Treatment Kit

See Section 4.7.

7.2 Sweat Collection

The following supplies are needed to perform sweat testing on gauze or filter paper, or in microbore tubing.

7.2.1 On Gauze or Filter Paper

- Gauze squares or filter paper: These should be low in sodium and chloride content. If filter paper is used, it should be of a type sufficiently absorbent to collect all of the stimulated sweat. Use the same size gauze or filter paper for stimulation and collection.
- Weighing vials: Disposable plastic vials with a snugly fitting cap, sufficient to hold 10 mL of diluent.
- Two pieces of paraffin wax film cut into squares measuring 7.6 cm (3.0 in).
- Waterproof surgical tape [2.5 cm (1.0 in) wide] or disposable stretch bandage and paper tape.
- Powder-free gloves or forceps.

7.2.2 In Microbore Tubing

- Sweat collector with attachment strap and optional clip
- Powder-free gloves (optional)
- Microsample cups with snugly fitting caps
- Disposable stretch bandage (optional)
- Collector volume calibration chart (supplied by the manufacturer).

7.3 Chloride Determination by Chloridometer

- Acid diluent: acetic acid/nitric acid: 250 mL glacial acetic acid and 16 mL concentrated nitric acid

Add 250 mL of glacial acetic acid and 16 mL of concentrated nitric acid to 1,000 mL of distilled or deionized water, and mix thoroughly. Store in a glass bottle. The diluent is stable for six months at room temperature.

- Gelatin reagent: Gelatin reagent can be obtained commercially in a preparation containing a pH indicator. Follow the manufacturer's recommendations for preparation and storage.

- Chloride calibrators, 1.0 mmol/L and 50 mmol/L: The chloride calibrators can be prepared in-house or purchased commercially.

  To prepare a 1.0 mmol/L-chloride calibrator, weigh 0.0584 g of desiccated NaCl and add sufficient volume of distilled or deionized water to bring the volume to 1 L.

  To prepare a 50 mmol/L-chloride calibrator, weigh 2.9220 g of desiccated NaCl and add sufficient volume of distilled or deionized water to bring the volume to 1 L.

  Document the verification of newly prepared calibrators against existing calibrators.

  Store the calibrators in a tightly stoppered glass bottle at 4 °C. The calibrators are stable for three months or according to the manufacturer's recommendations. Bring them to room temperature before use.

- Low- and high-chloride controls: Recommended concentration for the low control is in the range of 10 to 30 mmol/L with a high control of 100 mmol/L chloride. Make the controls up separately from the reagents used to calibrate the chloridometer, and store them in tightly stoppered glass bottles at 4 °C. The controls are stable for three months or according to the manufacturer's recommendations. Bring them to room temperature before use.

  The chloride controls may be prepared in-house or purchased commercially.

  To prepare a 10 mmol/L-chloride control, weigh 0.5844 g of desiccated NaCl and add a sufficient volume of distilled or deionized water to bring the volume to 1 L.

  To prepare a 100-mmol/L chloride control, weigh 5.8441 g of desiccated NaCl and add a sufficient volume of distilled or deionized water to bring the volume to 1 L.

  To assign control values, assay the controls once a day for 20 days and calculate the mean and standard deviations.

8 Procedures

8.1 Sweat Stimulation and Collection

8.1.1 Effects of Patient Age

During the first 24 hours after birth, sweat electrolyte values are transiently elevated; up to 25% of normal newborns show a sweat sodium concentration greater than 65 mmol/L.[16]

Beginning on the second day after birth, there is a rapid decline in sweat electrolyte concentration, and an elevated value can be used to confirm the diagnosis of CF.[16] Therefore, it is recommended that sweat testing not be performed on infants younger than 48 hours of age. Thereafter, if an adequate sweat sample is obtained, the results can be used to confirm or exclude the diagnosis of CF. Clinical experience

(18) Remove the protective backing from the paraffin wax film. Lay the first piece of the paraffin wax film with the uncontaminated side up on a clean, dry paper towel. Similarly, overlay the first piece with the second piece of paraffin wax film, uncontaminated side up. This will ensure that a clean surface comes into contact with the patient's skin. Keeping the preweighed vial in the carrier glove, carefully remove the collecting gauze from the preweighed vial and place it on top of the paraffin wax film. Place the paraffin wax film and gauze over the site of the pilocarpine iontophoresis, with the gauze contacting the skin.

(19) Secure the paraffin wax film with two strips of waterproof tape on all sides or wrap the site with a disposable stretch bandage and secure it with paper tape. Create an airtight seal between the skin, gauze, and paraffin wax film sheets.

(20) Record either the time of initiation or completion of collection and note this on the laboratory requisition.

(21) Repeat the entire procedure on the patient's opposite extremity, using the same size electrodes on both sites. To ensure consistency of collection, the entire collection procedure should be performed by the same trained laboratory personnel.

(22) Allow the sweat to collect for 30 minutes. Extending the collection time will not significantly increase the sweat yield and can potentially lead to false results.

(23) At the end of the 30 minutes, remove and discard the disposable stretch bandage or waterproof tape.

(24) Gently blot the paraffin wax film against the gauze to collect any condensate that could have formed on the lower surface of the paraffin wax film during collection. Failure to collect this condensate can result in false-positive values.

(25) Lift the corners of the paraffin wax film and carefully remove the paraffin wax film and gauze together. Do not touch the gauze with the gloves; rather, use the paraffin wax film as the gauze cover.

(26) Quickly (to minimize evaporation) transfer the gauze into the vial from which it was removed; guide the gauze with the paraffin wax film. Do not place the paraffin wax film in the vial with the gauze; discard the paraffin wax film after using it to guide the gauze into the vial. The gauze, the paraffin wax film, and the preweighed vial must never come into contact with the hands or be contaminated in any way. Always use powder-free gloves when handling the collection materials. Always keep the preweighed vial and lid inside the carrier glove.

(27) Reweigh the vial promptly and record the weight. At least 0.075 g of sweat should be collected using 5.1 x 5.1-cm (2 x 2-in) gauze. (Refer to Section 8.1.5.) To assure analytical consistency, the same trained laboratory personnel who preweighed the vial should reweigh the vial after sweat collection.

8.1.3.1 Procedural Notes and Precautions

Refer to Section 11.4 for possible sources of error.

(1) In place of wearing powder-free gloves to avoid contamination, the weighing vial, collecting gauze, and paraffin wax film can be handled with forceps that have been rinsed with distilled or deionized water and dried.

(2) Filter paper can be used in place of gauze for stimulating and collecting the sweat.

(3) To minimize evaporation of the sweat sample:

- Use two strips of waterproof adhesive tape on all sides of the paraffin wax film, or wrap with a disposable stretch bandage to produce an airtight seal.
- After collection, quickly transfer the gauze or filter paper to the weighing vial and reweigh the vial.

(4) To minimize contamination:

- Use gauze and/or filter paper that is low in sodium and chloride content.
- Wash and dry the patient's skin thoroughly.
- Do not directly handle the weighing vial, the paraffin wax film, the collection site, or the collection gauze with the fingers. Always use forceps or powder-free gloves.

8.1.4 Use of Microbore Tubing Collector

Follow the manufacturer's instructions for use of the power supply and collection system.

8.1.4.1 Procedural Notes and Precautions

Refer to Section 11.4 for possible sources of error.

(1) Carefully inspect the pilocarpine-containing gel discs to ensure that there are no physical defects.

(2) Microbore tubing collectors are individually wrapped. Avoid touching the concave collecting surface with the fingers (or gloves), because this will contaminate the surface and possibly lead to a falsely elevated result.

(3) Be sure that the extremity is thoroughly washed and dried before applying the microbore tubing collector.

(4) To ensure an efficient sweat collection, fasten the microbore tubing collector to the extremity with firm strap pressure. Test for proper attachment after sweat appears in the tubing (refer to the manufacturer's instructions).

(5) Do not attempt to remove the entire collector assembly from the patient's extremity before separating the microbore tubing from the main body. Transient vacuum can cause a loss of part or all of the specimen.

(6) Sweat should be collected for 30 minutes. The minimum sample volume collected should be 15 µL. (Refer to Section 8.1.5.)

(7) For quality assurance, it is recommended that the procedure be repeated by collecting and analyzing a sample from the opposite extremity.

(8) Before testing another patient, visually confirm that the nippers and sweat-dispensing needle have not become contaminated with sweat from the previous test.

8.1.5 Sample Requirements

Because sweat electrolyte concentration decreases at low sweat rates, an accurate sweat test requires the measurement of sweat electrolytes from maximally stimulated sweat glands.[14] Measuring sweat electrolytes at low sweat rate could lead to false-negative results.[14] In addition, evaporation becomes a more significant problem with smaller samples.[11]

The minimum acceptable sweat volume and/or weight depends on the size of the electrode used; the type and size of the collecting material used (gauze, filter paper, or microbore tubing); and the length of time the sweat is collected. The sweat rate should exceed 1g/m^2/min, which, in general, corresponds to a minimum sample weight of about 0.075 g of sweat collected on 5.1 x 5.1-cm (2.0 x 2.0-in) gauze or filter paper and about 15 µL of sweat collected in microbore tubing in 30 minutes. Samples less than 0.075 g or 15 µL should not be analyzed, nor should insufficient sweat samples be pooled to achieve the weight/volume requirement. It is imperative that the minimum sample amount be based on a specific collection time derived from the rate equation. For example, if a laboratory extends the collection time beyond 30 minutes, a larger volume of sweat will be required. Extending the collection time beyond 30 minutes is not recommended.

The sample volume requirement is also dependent on the test volume required by the analytical method. For example, a sample size of 50 µL collected from microbore tubing is required for the chloride determination described in Section 8.2.

In patients from whom an adequate sweat sample is not obtained, repeat testing may be carried out as soon as is practical, because the rate of sweating can vary from day to day. Thermal stimulation over the iontophoresis site, use of a warmer for an infant (with careful monitoring of the infant's temperature), or feeding or nursing an infant during the collection period can increase sweat production. In cases in which an adequate sweat sample cannot be obtained from one site, testing can be repeated at another site, but inadequate samples from several sites cannot be pooled for analysis.

To ensure adequate sweat production:

- Check the polarity of the electrodes. Pilocarpine will not be delivered from the negative electrode.
- Check that the pilocarpine is within the expiration date.
- To avoid bridging, check that the skin between the electrodes remains dry during iontophoresis. If the skin is wet with reagent between the two electrodes, the current will "bridge" between them in a path of low resistance, and pilocarpine will not be transported into the sweat glands.

8.2 Measurement: Chloride by Titration with a Chloridometer

8.2.1 Principle

The chloride concentration in sweat can be determined from a sample by titration using a chloridometer. Sweat samples collected on gauze or filter paper require elution with a diluent before analysis.

8.2.2 Procedure

The analytical method should be systematically and periodically validated by the laboratory for accuracy, precision, and reportable range using specimens corresponding to the volume and concentration of patient sweat samples.

(1) Clean and maintain the electrodes on the chloridometer according to the manufacturer's recommendations.

(2) Analyze the 1.0- and 50.0-mmol/L calibrators and the low and high controls at room temperature.

(3) Do not allow any portion of the materials used in the analysis to contact the hands. Use powder-free gloves.

8.2.2.1 Sweat Collected on Gauze or Filter Paper

(1) Rinse with distilled or deionized water and dry several 1-mL volumetric pipets or use disposable pipets.

(2) Place a piece of gauze the same size as the collecting gauze into a clean, plastic vial with a cap. Label this vial "Gauze Blank." The source for this gauze (or filter paper) blank should be the same as that used for the sample collection.

(3) Prepare "control" vials. Weigh two vials (labeled "Low Control" and "High Control") that contain gauze and record their weights. Add 100 µL of the controls to the appropriately labeled vials. Reweigh the vials, record the weights, and subtract the difference. This difference should be 0.10 g (about ±2%). If this value deviates significantly from 0.10 g, the accuracy of pipets and weighing should be investigated.

(4) Using an automatic pipettor, add 8 mL of distilled or deionized water to the patient vials, the gauze blank vial, and the control vials. Recap the vials and shake them for 1 minute. Allow the vials to sit at room temperature for at least 15 minutes.

(5) At the end of the 15 minutes, use a pipet to press the sweat/diluent eluate from the gauze and remove the gauze from the vials. Use the eluate as the test solution in Step 6. If sweat is collected on filter paper, the vials should not be shaken and the elution time should be increased to 40 minutes. Mix the eluate before analysis.

(6) Setup the chloride titration vials in duplicate as shown in Table 1, or follow the manufacturer's instructions. The solution's acidity is indicated by the presence of a pink color when four drops of gelatin reagent are added to the diluted sample. Do not titrate unless the pink color is present.

(7) For chloridometer set-up and analysis, follow the manufacturer's instructions and titrate the blanks, calibrator, and test/controls. Record the titration values.

(8) Perform the calculations in Section 8.2.3.1.

Table 1. Chloride Titration Vials

	Blank	Gauze Blank	Calibrator	Test/ Control
Acid diluent	3 mL	3 mL	3 mL	3 mL
Distilled or deionized water	1 mL	—	—	—
1.0 mmol/L Cl^- calibrator	—	—	1 mL	—
Gelatin reagent	4 drops	4 drops	4 drops	4 drops
Test solution from vials	—	1 mL	—	1 mL

8.2.2.2 Sweat Collected in Microbore Tubing

For sweat collected in microbore tubing, if the specimen volume is less than 50 µL, method validation is required for the analytical system.

(1) Use a pipette to transfer 4.0 mL of the acid diluent into each of the following labeled titration vials: "Blank," "50.0-mmol/L Calibrator," "Low Control," "High Control," and "Patient." Add four drops of gelatin reagent to each of the vials.

(2) To the calibrator, controls, and patient titration vials, add 50 µL of the appropriate sample. If the specimen volume is less than 50 µL, method validation is required.

(3) Titrate the blank, calibrator, controls, and patient samples according to the manufacturer's recommendation. Record the results.

(4) Repeat steps (2) through (4) and calculate the average of the values obtained.

(5) Perform the calculations in Section 8.2.3.2.

8.2.3 Calculations

8.2.3.1 Sweat Collected on Gauze or Filter Paper

For sweat collected on gauze or filter paper, calculate the average of the duplicate readings for each vial, then perform the following calculation:

$$\frac{\text{Test} - \text{Gauze Blank}}{\text{Calibrator} - \text{Blank}} \bullet \frac{\text{mL of Diluent (8mL)} + \text{g of Sweat}}{\text{g of Sweat}} \bullet \frac{1.0\ \text{mmol/L}}{\text{(Calibration Concentration)}} = \text{mmol/L Sweat } Cl^- . \quad (1)$$

Table 2 and the following equations are examples of how this calculation is performed:

Table 2. Example Data

	Data	Mean
Calibrator	140/142	141
Blank	40/44	42
Test	58/62	60
Gauze blank	49/49	49
Gram of sweat	0.1004	

$$\frac{60-49}{141-42} \bullet \frac{8+0.1004}{0.1004} \bullet 1 = \text{mmol/L};$$

$$\frac{11}{99} \bullet \frac{8.1004}{0.1004} \bullet 1 = 9 \text{ mmol/L sweat } Cl^{-}.$$

8.2.3.2 Sweat Collected in Microbore Tubing

For sweat collected in microbore tubing, calculate the concentration of chloride in mmol/L according to the following equation:

$$\frac{(\text{Test} - \text{Blank})}{(\text{Calibrator} - \text{Blank})} \bullet \frac{50.0 \text{ mmol/L}}{(\text{Calibrator Concentration})} = \text{mmol/L Sweat } Cl^{-}. \quad (2)$$

8.2.3.3 Reporting Results

(1) Round results off to the nearest whole number, and have the calculations reviewed by a supervisor.

(2) Compare the bilateral sweat chloride values. They should agree within 10 mmol/L for values less than or equal to 60 mmol/L, and within 15 mmol/L for values greater than 60 mmol/L.

(3) Check that the controls are within the acceptable range (±2 SD).

8.3 Nonselective Methods for Measuring Electrolytes in Sweat

8.3.1 Conductivity

Conductivity is the property of a solution that allows it to conduct a current. The conductivity depends on the concentration and mobility of the ions in the solution and represents a nonselective measurement of ions.[22-24] Sweat conductivity concentration is not equivalent to sweat chloride concentration because of other ions in sweat such as bicarbonate and lactate. On average, sweat conductivity is approximately 15 mmol/L higher than sweat chloride.[25] Sweat-conductivity-measuring instruments are available from several manufacturers. However, some of them lack stability and temperature control, introduce air bubbles, and allow for sample evaporation. A conductivity instrument designed specifically for use with the microbore tubing collector that addresses the above concerns has been approved by the Cystic Fibrosis Foundation as a screening method but only outside of accredited Cystic Fibrosis Care Centers.[8,26,27] A sweat conductivity decision level of greater than or equal to 50 mmol/L (equivalent sodium chloride, NaCl) is recommended by the Cystic Fibrosis Foundation, and the patient should be referred for a quantitative sweat chloride test.[8]

8.3.2 Osmolality

Osmolality of sweat reflects the total solute concentration in millimoles per kilogram of sweat. The sweat osmolality measures the total cations and anions along with other solutes such as urea and amino acids. The latter solutes are present in small concentrations in sweat. It is necessary to use an osmometer capable of measuring undiluted micro-samples of sweat. Dilutions must not be used in connection with sweat osmolality determinations, because the osmotic coefficient changes with the concentration of the specimen, and this would give rise to measurement error.[28] Trials with hospital patients have shown that the reference interval for sweat osmolality in children is approximately 50 to 150 mmol/kg.[28-31] Children with CF, confirmed as positive with a quantitative chloride measurement, have sweat osmolality values above 200 mmol/kg; values between 150 and 200 mmol/kg are equivocal.[28-31]

8.4 Sodium

Sweat sodium is elevated in CF but provides less discrimination when compared to chloride in diagnosis. It is recommended that sodium not be used alone for the diagnosis of CF. A few laboratories measure sodium in addition to chloride for quality control purposes (see Section 10.4) and/or as an adjunct in assessing the atypical patient. Genotyping and nasal transepithelial potential differences are often more diagnostically useful than sweat sodium when testing the atypical patient for CF.[1] Procedures for sweat sodium are found in Appendix F.

9 Labeling of Containers

9.1 Reagents, Calibrators, and Controls

Label reagents, calibrators, and controls according to contents, date of preparation, date placed in service, storage, expiration date, and initials of laboratory personnel.

9.2 Sweat Collected on Gauze or Filter Paper

Label the body and cap of the weighing vial with a number or letter coded to represent the test date, patient identification information, and the designation "left" or "right" prior to the initial weighing.

9.3 Sweat Collected in Microbore Tubing

Label the microsample cup with a number or letter coded to represent the test date, patient identification information, and the designation "left" or "right."

10 Quality Control

Little has been published about quality control (QC) procedures when testing sweat for electrolytes. Few, if any, of the published methods refer to this important issue. However, a variety of approaches to this problem are presented below.

10.1 Procedures

The optimal approach is to process QC materials in exactly the same way as the unknown specimen. This is particularly important when the sweat is collected onto preweighed filter paper or gauze. One way to achieve this is for the person collecting the sweat to have available a solution of electrolyte (e.g., sodium and/or potassium chloride) of known composition. Immediately after obtaining the patient sample, 100 µL of the control solution is placed onto another preweighed filter paper or gauze and processed in parallel with the unknown. The control gauze or filter paper should show a weight increase of 0.1 g.

Both a "low" and a "high" QC solution should be processed simultaneously with each patient sample or batch of patient samples. The QC materials should be unique when compared to the calibrators used to calibrate the analytical instrument. The advantage of this approach is that all aspects of the process, with the exception of the sweat collection, are evaluated (i.e., weighings, dilutions, measurements of the analyte, and calculations). Direct analysis of QC material for the analyte(s) of interest is insufficient to ensure that all aspects of the test are adequately controlled. However, this is acceptable if an undiluted specimen is obtained using a microbore tubing collector.

10.2 Tolerance Limits

Tolerance limits should be established for the QC values and the values recorded and reviewed regularly in accordance with existing standards of laboratory practice. Greater imprecision can be expected when smaller amounts of sweat are obtained (e.g., 0.075 to 0.1 g) and when the sweat chloride concentration is low.

10.3 Collection in Duplicate

There is generally a good correlation between the amount of sweat collected at different sites from the same patient. Criteria for acceptable agreement between analyte values from two different collection sites are not precisely defined, but chloride values usually agree within a few mmol/L of each other with tolerance limits of within 10 mmol/L for values less than or equal to 60 mmol/L, and within 15 mmol/L for values greater than 60 mmol/L.

10.4 Collection and Analysis

Laboratories may choose to analyze sodium along with chloride as a mechanism of quality control. Both analytes should be proportionally increased or decreased. Discordant values can indicate problems with collection or analysis. Generally, sweat chloride and sodium concentrations agree within 15 mmol/L of each other. In most CF patients, the Cl:Na ratio is greater than one.[1]

Sweat chloride or sodium concentrations >160 mmol/L are not physiologically possible and therefore the patient should be retested.[32]

Ideally, the sweat collection and analysis should be performed by the same person. The test should be performed only by experienced, well-trained persons who perform a sufficient number of tests to maintain proficiency. In many laboratories, this can be accomplished by limiting the performance of the test to a small number of trained laboratory personnel.

10.5 Quality Assurance

10.5.1 Internal Quality Assurance

The amount of sweat collected should be included on the laboratory chart report. When an insufficient amount of sweat is obtained, it is preferable to discard the sweat collected without any attempt at analysis.

The laboratory report should specifically state what analyte is being measured in the sweat test and apply the analyte-specific reference range. For example, it is important to apply sweat chloride reference ranges to chloride determination and apply distinctly different reference ranges if sweat conductivity is measured.

The amount of sweat collected should routinely be monitored to determine the proportion of patients from whom adequate sweat collection cannot be obtained. This proportion will vary depending on the patient population. Unless many of the patients tested are very young infants (less than one month) the

proportion of inadequate collections should not exceed 5%. If a proportion substantially greater than this is observed (particularly if it represents a change from that previously observed), the cause should be investigated.

It is recommended that all positive or equivocal sweat chloride results be followed by: (a) repeat collection and determination of sweat chloride, and/or (b) gene mutation determination. It is also recommended that all negative sweat chloride results be followed by repeat collection and analysis if clinical symptoms of CF persist and no other cause has been determined.

10.5.2 External Quality Assurance

Laboratories should participate in some type of external quality assurance such as proficiency testing programs (e.g., in the U.S. the College of American Pathologist and in the U.K. the National External Quality Assessment Schemes) to assess testing accuracy. Participants should be aware that the programs primarily assess the analytical portion of the sweat test.

11 Evaluation of Results

11.1 Reference Values

The results of quantitative measurement of sweat chloride in patients with CF, healthy siblings, and controls are shown in Figure 1. In general, sweat chloride concentrations less than 40 mmol/L are considered normal, values between 40 to 60 mmol/L are borderline, and sweat chloride concentration greater than 60 mmol/L are consistent with the diagnosis of CF.[1] Sweat chloride results should always be evaluated in light of the patient's clinical course and with regard to the patient's age. Data from a newborn screening program suggests that sweat chloride concentrations over 40 mmol/L in young infants appear to be very suggestive of a CF diagnosis.[33] Electrolytes in sweat will continue to be increased in patients with CF, regardless of treatment. There is no direct correlation between electrolytes in sweat and the severity of the disease; however, CF patients with pancreatic sufficiency tend to have lower sweat electrolyte values.[34]

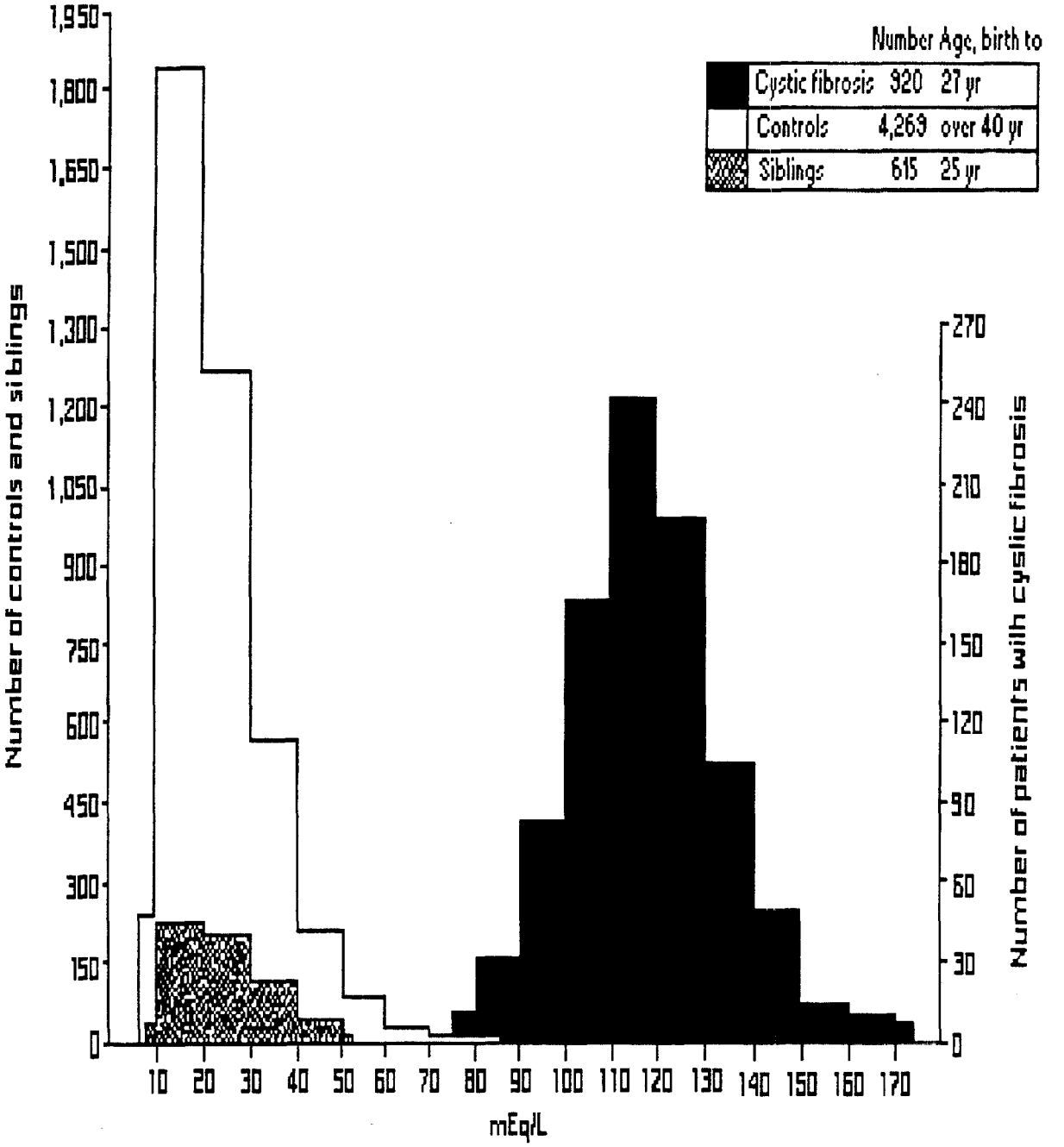

Figure 1. Chloride Concentrations in Patients with CF, Healthy Persons, and Healthy Siblings of Patients with CF. A chloride concentration greater than 60 mmol/L (60 mEq/L) is consistent with the diagnosis of cystic fibrosis. The concentration in most healthy persons is less than 40 mmol/L (40 mEq/L). Healthy siblings of patients with CF have normal values. From Shwachman H, Mahmoodian A. Pilocarpine iontophoresis sweat testing results of seven years' experience. In Rossi E, Stoll E, eds. *Modern Problems in Pediatrics*. Basel/NY: S. Karger AG. 1967;10:158-182. Reprinted with permission.

11.2 Diagnostic Criteria

Results from the measurement of chloride concentrations in sweat should be interpreted in relation to the patient's clinical picture by a physician knowledgeable about CF. The test results should be consistent with the clinical picture; no single laboratory result is sufficient to establish or rule out the diagnosis of CF. The criteria for the diagnosis of CF include: the presence of one or more characteristic phenotypic features, or a history of CF in a sibling, or a positive newborn screening test result; and an increased sweat chloride concentration by pilocarpine iontophoresis on two or more occasions, or identification of two CF mutations or demonstration of abnormal nasal epithelial ion transport.[1] Two percent of patients with documented CF have sweat chlorides less than or equal to 60 mmol/L, and in these patients the diagnosis of CF was based on genotyping, nasal potential difference measurements, and clinical presentation.[35] Therefore, a normal sweat chloride value cannot be used as the sole criterion for exclusion of a CF diagnosis.

11.3 Other Diseases Associated with Elevated Sweat Electrolyte Concentrations

A variety of diseases other than CF can be associated with moderately elevated concentrations of sweat electrolytes.[36] (Refer to Appendix D.) However, with few exceptions, these conditions do not represent a problem in differential diagnosis.

11.4 Sources of Error

Sweat electrolyte values can vary depending on the method of stimulation of sweating, the volume of sweat collected, the sweat secretory rate, salt intake, and nutritional and hydration status.[37] However, it is rare for these factors to interfere with the diagnostic validity of the results. In general, false-positive results are more frequently seen than false-negative results.[2,38,39] Most errors are caused by use of unreliable methodology, inadequate sweat collection, technical errors, and misinterpretation of the results. Problems are also attributable to inexperienced laboratory personnel and lack of appropriate quality assurance.

Because of methodological problems, confirmation or rejection of the diagnosis of CF should only be based on test results carried out using the quantitative methodology described in this document. Direct reading *in situ* tests using ion-selective electrodes or older electrical conductivity measurements are not acceptable as diagnostic tests.[2,3,38,39] With direct reading procedures, the amount of sweat collected is not measured, and an adequate sample cannot be assured. Unheated cup collectors are unsatisfactory because of condensation.[3,7]

Technical problems associated with the determination of sweat chloride include:

- failure to obtain an adequate sweat sample;
- skin contamination by salt-containing materials;
- failure to adequately dry the patient's skin before sweat collection;
- evaporation of the sweat sample during collection, transfer, and transport;
- failure to include condensate in the sweat sample when using gauze or filter paper; and
- errors in sample weighing, dilution, elution, electrolyte determination, and result computation.

Errors in interpretation include:

- establishment of a diagnosis of CF on the basis of a single positive test result;
- failure to repeat a test giving borderline results; and
- failure to repeat tests in patients with a negative result but a clinical picture highly suggestive of CF.

False-negative sweat electrolyte results have been reported in the presence of edema and hypoproteinemia.[40, 41] In such cases, it is mandatory to repeat the test after resolution of the edema.

For additional considerations in the quantitative measurement of sweat chloride, refer to Appendix E.

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Appendix A. Indications for Sweat Testing

Pulmonary and Upper Respiratory Tract Indications	Gastrointestinal Indications	Metabolic and Other Indications
Chronic cough	Meconium ileus	Positive family history
Recurrent or chronic pneumonia	Meconium plug syndrome	Failure to thrive
Wheezing*	Prolonged neonatal jaundice	Salty taste to skin
Hyperinflation*	Steatorrhea	Salt crystals on skin
Tachypnea*	Rectal prolapse	Salt-depletion syndrome
Retractions*	Mucoid impacted appendix	Metabolic alkalosis
Atelectasis (especially of the right upper lobe)	Late intestinal obstruction	Hypoprothrombinemia
Bronchiectasis	Recurrent intussusception	Vitamin A deficiency (bulging fontanelle is a key sign)
Hemoptysis	Cirrhosis	Azoospermia
Mucoid pseudomonas infection	Portal hypertension	Absent *vas deferens*
Nasal polyps	Recurrent pancreatitis	Scrotal calcification
Pansinusitis		Hypoproteinemia
Digital clubbing		Edema

* If persistent or refractory to usual therapy.

Appendix B. Current Density

Different power sources, both commercial and homemade, are currently in use. The current density, defined as ampere per square meter of electrode surface delivered, is important. (This can be expressed in mA/cm^2.) An excess current density can cause discomfort and burns.

It has been found that a current of 4 mA through a 5.1 x 5.1-cm (2.0 x 2.0-in) gauze square rarely causes difficulties. If this current is applied for 5 minutes, theoretically 3.4 mg of pilocarpine can be delivered.

$$P = \frac{itMW}{F} = \frac{4 \cdot 300 \cdot 271}{} = 3.370 \text{ mg.}$$

where:

P = mg pilocarpine nitrate delivered to the skin surface,
i = 4.0 mA,
t = 300 seconds,
F = Faraday constant = 96,489 coulombs/mole, and
MW = molecular weight = 271.

Here the current density is 0.16 mA/cm^2, the systemic adult dose of pilocarpine is 10 mg, and iontophoresis is probably about 50% efficient. This theoretical dose has not been found to cause any reactions in infants or children.

Electrodes smaller than 5.1 x 5.1 cm (2.0 x 2.0 in) give better skin contact, and higher current densities may be used. The microbore tubing collector system uses an electrode of 6.25 cm^2 (2.5 in^2) and a current of 1.5 mA. The current density is 0.24 mA/cm^2.

Appendix C. Concentration of Pilocarpine Nitrate

When an excess of pilocarpine is placed on the positive electrode, the amount of pilocarpine delivered to the skin is controlled by the current used and the time of iontophoresis. An excess of current would have obvious disadvantages. As mentioned in Appendix B, a current of 4 mA for five minutes can theoretically deliver 3.37 mg of pilocarpine. A 5.1 x 5.1-cm (2.0 x 2.0-in) gauze square is thoroughly wet by 2 or 3 mL of solution. If a 0.2% solution (0.2 g/100 mL) is used, 2 mL will contain:

$$\frac{200\ \text{mg}}{100\ \text{mL}} \bullet 2\ \text{mL} = 4\ \text{mg pilocarpine nitrate.}$$

A stronger solution would not be harmful; in practice, 0.2 to 0.5% solutions are used.

Appendix D. Diseases or Conditions Other Than Cystic Fibrosis Associated with an Elevated Sweat Electrolyte Concentration

Anorexia nervosa

Atopic dermatitis

Autonomic dysfunction

Ectodermal dysplasia

Environmental deprivation

Familial cholestasis (Byler's disease)

Fucosidosis

Glucose-6-phosphate dehydrogenase deficiency

Glycogen storage disease type 1

Hypogammaglobulinemia

Klinefelter's syndrome

Long-term prostaglandin E_1 infusion

Mauriac's syndrome (malnutrition of)

Mucopolysaccharidosis Type I

Nephrogenic diabetes insipidus

Nephrosis

Protein-calorie malnutrition

Pseudohypoaldosteronism

Psychosocial failure to thrive

Untreated adrenal insufficiency

Untreated hypothyroidism

Appendix E. Additional Considerations with Sweat Electrolyte Determination

E1 Transient Elevation in Sweat Electrolyte Concentrations

Evidence exists that there can be physiological variability of sweat electrolyte concentrations over time and that there can be transient elevation of sweat electrolyte values in healthy persons.[1] Transient elevation of sweat electrolyte concentrations has also been reported in patients with anorexia nervosa[2] and in association with environmental deprivation and psycho-social failure to thrive.[3]

E2 Borderline Sweat Chloride Concentrations

Patients who have persistent borderline sweat chloride concentrations present a difficult diagnostic challenge. In such cases, it is important to carry out CF mutational determination and quantitative assessment of exocrine pancreatic function. Ancillary findings, such as urogenital abnormalities including azoospermia in postpubertal men, isolation of a mucoid strain of *Pseudomonas aeruginosa* from the respiratory tract, and radiographic evidence of pansinusitis may be helpful. Measurement of nasal transepithelial potential differences and response to amiloride and isoproterenol can also be helpful in such cases.[4,5] Approximately 1% of patients with CF exhibit sweat chloride concentrations persistently in the range of 40 to 60 mmol/L in association with chronic pseudomonas bronchitis and preservation of pancreatic function.[6]

E3 Sweat Chloride Concentrations in Adults with CF

While the majority of CF patients are diagnosed within the first year of life, the diagnosis is being made with growing frequency in adults. Patients diagnosed after 18 years of age tend to have milder CF mutations, resulting in less severe pulmonary disease and pancreatic sufficiency. Such patients may have normal or borderline sweat chloride concentrations which confound the diagnosis. In these situations, nasal potential differences can be useful in demonstrating CFTR dysfunction.[7]

E4 Repeat Sweat Chloride Determination

E4.1 Indications

- All positive sweat chloride results should be repeated and/or confirmed with mutation determination; the diagnosis of CF should never be based on a single positive test.

- All borderline sweat chloride results (chloride concentration 40 to 60 mmol/L) should be repeated; if results remain in a borderline range, additional ancillary tests can be helpful (see Section E2).

- The collection and determination of sweat chloride should be repeated in patients with "confirmed" CF who do not follow an expected clinical course.[8-10] As patients are followed, the clinical, laboratory, and chest radiograph findings should be consistent with the diagnosis of CF. It is especially important to re-evaluate those patients in which: the initial diagnosis was suggested primarily on the basis of failure to thrive or a positive family history; the clinical features prompting the initial sweat analysis disappear; the course is consistent with asthma without suppurative lung disease; or there is a normal growth pattern without evidence of digital clubbing, pseudomonas colonization, or typical chest radiograph changes.[9]

E4.2 Timing of Repeat Testing

Repeat sweat collection and analysis can be carried out at any time after the initial testing but preferably at a time when the patient is clinically stable, well-hydrated, free of acute intercurrent illness, and not receiving mineralocorticoids.

References to Appendix E

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3. Christooffel KS et al. Environmental deprivation and transient elevation of sweat electrolytes. *J Pediatr.* 1985;107:71-76.

4. Knowles MR, Pardiso AM, Boucher RC. *In vivo* nasal potential difference: techniques and protocols for assessing efficacy of gene transfer in cystic fibrosis. *Hum Gene Ther.* 1995;6: 445-455.

5. Wilson DC, Ellis L, Zielenski J, Corey M, IP Wr, Tsui L-C et al. Uncertainty in the diagnosis of cystic fibrosis: possible role of in vivo nasal potential difference measurements. *J Pediatr* 1998;132: 596-599.

6. Stern RC et al. Intermediate-range sweat chloride concentration and Pseudomonas bronchitis. *JAMA.* 1978;239:2676-2680.

7. Cystic Fibrosis Foundation. Cystic Fibrosis Foundation Consensus Report: Diagnosis of Cystic Fibrosis in Adults; 1999.

8. Rosenstein BJ, Langbaum TS. Misdiagnosis of cystic fibrosis. *Clin Ped.* 1987;26:78-82.

9. Smalley CA, Addy DP. Does that child really have cystic fibrosis? *Lancet.* 1978;2:415-416.

10. Shaw NJ, Littlewood JM. Misdiagnosis of cystic fibrosis. *Arch Dis Child.* 1987;62:1271-1273.

Appendix F. Sweat Sodium Determination

F1 Flame Photometer

Use a flame emission photometer, preferably with a diluter, to determine sweat sodium concentration. Flame emission photometers employ an internal calibrator (commonly lithium or cesium salts).

F1.1 Sodium Determination

- Diluent containing internal calibrator: The diluent can be purchased from the manufacturer or prepared in-house according to the manufacturer's recommendations.

- Propane: Instrument-grade propane supplied in disposable tanks should be available.

- Sodium Calibrators: Prepare 10-, 20-, and 40-mmol/L sodium calibrators.

  The calibrators can be prepared in-house or purchased commercially.

  To prepare a 10-mmol/L sodium calibrator, weigh 0.5844 g of desiccated NaCl and add a sufficient volume of distilled or deionized water to bring the volume to 1 L.

  To prepare a 20-mmol/L sodium calibrator, weigh 1.1688 g of desiccated NaCl and add a sufficient volume of distilled or deionized water to bring the volume to 1 L.

  To prepare a 40-mmol/L sodium calibrator, weigh 2.3376 g of desiccated NaCl and add a sufficient volume of distilled or deionized water to bring the volume to 1 L.

  Document the verification of newly prepared calibrators against existing calibrators.

- Low- and high-sodium controls: A recommended sodium concentration for the low control is in the range of 10 to 30 mmol/L with a high control of 100 mmol/L sodium. Prepare the controls separately from the reagents used to calibrate the flame photometer; store them in tightly stoppered glass bottles at 4 °C. The controls are stable for three months or according to manufacturer's recommendations. Bring them to room temperature before use.

  The sodium controls can be prepared in-house or purchased commercially.

  To prepare a 10-mmol/L sodium control, weigh 0.5844 g of desiccated NaCl and add a sufficient volume of distilled or deionized water to bring the volume up to 1 L.

  To prepare a 100-mmol/L sodium control, weigh 5.8441 g of desiccated NaCl and add a sufficient volume of distilled or deionized water to bring the volume to 1 L.

  To assign control values, assay the controls once a day for 20 days and calculate the mean and standard deviations.

F2 Measurement: Sodium by Flame Photometer

F2.1 Principle

The sodium concentration in sweat can be determined from a sample by flame photometry. Sweat samples collected on gauze or filter paper require elution with a diluent before analysis.

F2.2 Procedure

(1) For setup and analysis, follow the manufacturer's instructions.

(2) Using the diluent, dilute the 10-, 20-, and 40-mmol/L sodium calibrators using a ratio of 1:200.

(3) Aspirate the diluted 20-mmol/L sodium calibrator and adjust the instrument as necessary. Verify calibration by aspirating the 10- and 40-mmol/L diluted sodium calibrators.

(4) Do not allow any portion of the materials used in the analysis to contact the hands. Use powder-free gloves.

F2.2.1 Sweat Collected on Gauze or Filter Paper

If sweat is collected on gauze or filter paper:

(1) Label three vials: "low control," "high control," and "gauze/filter paper blank."

(2) Using forceps or powder-free gloves, add gauze or filter paper to each of the three vials.

(3) Add 50 µL of the appropriate control to the control vials.

(4) Add 10 mL of the diluent containing the internal calibrator to the control vials, the blank vial, and to the patient vial containing the collected sample. Recap the vials.

(5) If the sweat is collected on gauze, shake the patient vial, gauze/filter paper blank vial, and control vials for 1 minute. Allow the vials to sit at room temperature for 15 minutes.

(6) If the sweat is collected on filter paper, the vials should not be shaken but allowed to sit at room temperature for at least 40 minutes.

(7) At the end of the elution time, use a pipet to press the eluate from the gauze or filter paper and remove the gauze or filter paper from the vials. Mix the eluate before analysis.

(8) Analyze the eluted patient samples, controls, and blank according to the manufacturer's recommended procedure and record the results. Subtract the gauze/filter paper blank value from the patient value and the control values. The control values can be read directly from the flame photometer. The patient value is calculated using the appropriate equation.

F2.2.2 Sweat Collected in Microbore Tubing

If sweat is collected in microbore tubing:

(1) Use a pipet to transfer 2 mL of the internal calibrator diluent into each of the following sample vials labeled as follows: "Low Control," "High Control," and "Patient."

(2) Add 10 µL of the appropriate sample to each vial. Seal the vials with snugly fitting caps. Mix gently.

(3) Analyze the patient and control specimens according to the manufacturer's recommended procedure.

F2.2.3 Recalibration

If the sodium concentration of the patient's specimen or control is greater than 40 mmol/L, the flame photometer should be recalibrated and calibration verified with standards in the appropriate range. This is necessary, because some flame photometers are not linear throughout the reportable range of sweat sodium values.

F2.3 Calculations

F.2.3.1 Sweat Collected on Gauze or Filter Paper

For sweat collected on gauze or filter paper, calculate the patient result according to the following equation:

$$\text{Test (mmol/L)} - \text{Gauze Blank (mmol/L)} \bullet \frac{\text{mL of Diluent (10 mL)} + \text{g of Sweat}}{\text{g of Sweat}} \bullet \frac{1}{200} = \text{mmol/L Sweat } Na^{+}. \qquad (3)$$

F2.3.2 Sweat Collected in Microbore Tubing

For sweat collected in microbore tubing, the patient and control readout results directly represent the sodium concentration and no calculation is necessary.

F2.3.3 Reporting Results

(1) Record final results in whole numbers, and have the calculations reviewed by a supervisor.

(2) Check that the controls are within the acceptable range (±2SD).

Summary of Comments and Subcommittee Responses

C34-A: *Sweat Testing: Sample Collection and Quantitative Analysis; Approved Guideline—Second Edition*

General

1. Type II water is adequate in all areas where the document specifies Type I. Mainly because as soon as you apply Type I water to the patient's skin it is no longer Type I. Many places can't use Type I (e.g., pediatric clinics). Also, Type I water cannot be bottled.

- **This edition uses the term "distilled or deionized water" in place of Type I water.**

2. Two factors cannot be ignored in a rewrite of the guidelines: 1) Mention of genetic mutations in cystic fibrosis (CF); and 2) Mention of the "Undiagnosis" of CF (apparently due to mutations). The CF Foundation "Undiagnosis" about 0.5% of cases of CF annually (5 in 1,000). Mutations lead to at least three odd findings about sweat chloride in CF: 1) Normal chloride values at time of diagnosis of CF; 2) Borderline chloride values at time of diagnosis (Schwachman); and 3) Appearance of normal chloride values in the course of treatment after initial expected high values at diagnosis (undiagnosis).

- **Section 11.2 and Appendix E have been revised to include information concerning the diagnosis of CF to include genetic mutations.**

Section 6.1.2

3. Consider adding something about hybrid systems.

- **Hybrid systems exist when a laboratory uses one method for iontophoretic stimulation, and another method for collection. An example of a hybrid system would be using the coil collection electrodes and pilocarpine gel for iontophoresis and then collecting the sweat into gauze or filter paper. For consistent determination of the appropriate minimum sweat sample and to minimize the potential for evaporation, stimulation and collection systems should not be hybridized. If a laboratory collects sweat into coil collectors, then the appropriate iontophoretic system designed by the coil manufacturer should be employed. If a laboratory collects sweat onto gauze or filter paper, then copper or stainless steel electrodes described in Section 6.1.2 should be used.**

Section 8.1.3

4. The protocol appears not to have been modified to agree with the response to Comment 20. Furthermore there is still considerable concern regarding the use of latex gloves which generate static electricity. The use of forceps as suggested in the comment response (but not in the actual protocol) is hardly practical. Perhaps the committee could suggest cotton liners which will minimize problems with static electricity.

   Placement of preweighed vials in gloves for transport poses the same problem with static electricity. Plastic lunch bags will do the same. The use of a desiccator with desiccant will cause deposition of dust onto the vials. Therefore this does not seem to be a wise approach. What about the use of something like plastic seal-tight containers? Or perhaps the committee has another suggestion.

- **During the development of this guideline, this issue was discussed and the subcommittee at that time was not aware of any other laboratories with this problem. However, if this is a concern, then the use of glove liners is acceptable.**

5. On a site visit, it was observed that 2x2 gauze or filter paper wasn't being used for sweat collection. C34 doesn't explicitly state that 2x2 gauze or filter paper should be used, however, the CFF guidelines do. If this is important, please consider adding this to C34 during its revision.

- **In an effort to standardize sweat collection and employ a consistent minimum sweat amount, the Cystic Fibrosis Foundation requires that the CF Care Center choosing to collect into gauze or filter paper use 1 1/2 x 1/1/2 inch electrodes for stimulation and 2x2 inch gauze or filter paper for collection, as described in Sections 8.1 3 and 8.1.5.**

Section 8.1.5

6. It has long been known that patients with CF have normal or only slightly elevated concentrations of chloride in their sweat when sweat volumes are low (< 50uL). It is certainly my experience over a 26 year period using both the Gibson/Cooke and macroduct systems that low volumes following sweat gland stimulation often lead to sweat chloride concentrations in the normal to "grey zone" area in patients with CF. When on repeating the test and obtaining an adequate collection, (>50uL), the result is as expected, >60 mmol/L. I am therefore uncomfortable with the statement that only 15uL of sweat is needed. I agree with the comment that 0.075g of sweat is adequate when using gauze or filter paper. In my experience we need more than 15uL when collecting in microbore tubing. To prevent reporting of false negatives, I suggest that this volume requirement be stated to be >50uL.

- **The determination of the minimum acceptable sweat volume or weight is discussed extensively in Section 8.1.5. Because the area of stimulation and collection using coils is smaller than the area when using gauze or filter paper, the minimum sample requirements differ based on the collection technique used. The minimum acceptable volume for collection using the coil collectors (15 uL in 30 minutes) is based on the manufacturer's recommendation and is consistent with published work correlating the coil collector with collection into filter paper.[*] The committee is unaware of any published reports of false negative sweat tests using the 15 uL volume.**

Sections 8.2.2.1 and 8.3.2.1

7. The protocols given for measuring Na and Cl was different volumes of elution diluent, namely 8 mL distilled or deionized water versus 10 mL lithium nitrate. This indicates that one cannot use the same elutions for measuring both analytes. I don't believe this is what the committee intends. In addition the document does not address the issue of diluents sufficiently clearly.

   A difference has been found in the recovery on Na when eluting with water versus lithium nitrate. Elution with lithium nitrate appears to yield higher recovery of Na than with water. On the other hand Na continues to leach from the paper when it is left longer in contact with the water diluent. If a facility performs both Na and Cl analyses, the Cl should be performed first and the aliquots should be removed ASAP from the diluent if using water.

   The document does not give any information regarding the closeness of Na and Cl concentrations. Section 11.1 implies they are identical. How should the user address the values when they are not? Some information of this issue should be included.

- **Due to the declining role of sweat sodium for the diagnosis of CF and the declining number of laboratories continuing to analyze sweat sodium, the procedure has been moved to Appendix F. A common diluent could be used to elute the sweat samples in preparation for simultaneous sweat sodium and chloride determinations and then specific analytical reagents added with the**

[*] Hammond KB, Turcios NL, Gibson LE. Clinical evaluation of the macroduct sweat collection system and conductivity analyzer in the diagnosis of cystic fibrosis. *J Pediatr.* 1994;124:255-260.

final electrolyte concentration having been compensated for the dilutions performed. In patients with CF, sodium and chloride concentrations agree within 15 mmol/L and most often the chloride/sodium ratio is greater than 1.0.[†] Genotyping and nasal transepithelial potential differences are often more diagnostically useful than sweat sodium testing when evaluating patients with equivocal sweat chloride concentrations.

Section 8.4.1 (now 8.3.1)

8. The last sentence reads, "Any sweat conductivity result greater than or equal to 50 mmol/L (equivalent sodium chloride, NaCl) is considered positive and the patient should be referred for quantitative sweat electrolyte testing." I am concerned that the word "positive" as used here may be misinterpreted as meaning "diagnostic." It is my understanding that 50 mmol/L is considered to be a decision for referral for quantitative sweat electrolyte testing. I would suggest that we use the term "decision level" to avoid over-interpretation of conductivity results.

- **The suggested revision has been included in the second edition.**

Appendix B

9. Molecular weight for pilocarpine nitrate given in Appendix B appears to be incorrect.

- **The molecular weight for pilocarpine nitrate is 271, as stated in Appendix B.**

Summary of Comments and Subcommittee Response

10. The reply to Comment 27 misses the point. The user does not fiddle with the concentration of pilocarpine, which you state brings forth the maximum sweating rate. In all cases, the user must apply the sweat inducer as recommended. How much sweat is needed for chloride determination is an entirely different matter.

    Comment 27: The statement that samples less than .075 g or 15 µL should not be analyzed is not based on scientific study, and therefore should be softened to "samples less than 0.075 g or 15 µL are not commonly analyzed, hence probably should not be used. Nor should insufficient sweat samples be pooled, etc."

- **We reconsidered Comment 27 and believe it to provide a complete answer: "The subcommittee carefully considered these comments and recommends the sample size requirement of 15 µL and 75 mg based on the standard conditions described in the guideline, including electrode size, type and size of collection materials, and the length of collection time. The sweat rate should exceed 1 g/m^2/min, which corresponds to a minimum sample weight of about 75 mg of sweat collected on a 5.1 x 5.1-cm piece of gauze or filter paper and about 15 µL in microbore tubing in 30 minutes. Any change to the standard conditions described will affect the minimum sample requirement. Theoretically, it may be possible to collect a small amount of sweat from a small area of stimulation and still exceed 1 g/m^2/min rate; however, the possibility of evaporation increases as the sample volume decreases."**

[†] Rosenstein BJ, Cutting GR. The diagnosis of cystic fibrosis: A consensus statement. *J Pediatr.* 1998:132;589-595.

Summary of Delegate Comments and Responses

C34-A2: *Sweat Testing: Sample Collection and Quantitative Analysis; Approved Guideline—Second Edition*

1. In Section 8.1.3 (13) regarding iontophoresis, the maximum current should be based on the age of patient (i.e., <8 weeks of age: maximum current 1.5 mA and >8 weeks of age: maximum current 4.0 mA).

- **The project's management team is not aware of any published data to support a need for less than 4.0 mA current for infants less than eight weeks of age. However, currents less than 4.0 mA are acceptable as long as a sufficient sweat rate is achieved as indicated by the amount of sweat collected. See Section 8.1.5 for a discussion on sample requirements and Appendix B for a discussion on iontophoretic current density. (Webster HL. Laboratory diagnosis of cystic fibrosis. *CRC Crit Rev Clin Lab Sci.* 1983;18:313-338.)**

Related NCCLS Publications*

C3-A3 **Preparation and Testing of Reagent Water in the Clinical Laboratory; Approved Guideline—Third Edition (1997).** This document provides guidelines on water purified for clinical laboratory use, methods for monitoring water quality and testing for specific contaminants, and water system design considerations.

EP5-A **Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline (1999).** This document provides guidance for designing an experiment to evaluate the precision performance of clinical chemistry devices; recommendations for comparing the resulting precision estimates with manufacturer's precision performance claims and determining when such comparisons are valid; as well as manufacturer's guidelines for establishing claims.

EP6-P **Evaluation of the Linearity of Quantitative Analytical Methods; Proposed Guideline (1986).** This document contains a method for evaluating an instrument or quantitative analytical method on the basis of the manufacturer's linearity claim; and offers guidelines for manufacturer's use when stating a claim of an assay's linear range.

EP7-P **Interference Testing in Clinical Chemistry; Proposed Guideline (1986).** This guideline provides background information and procedures for characterizing the effects of interfering substances on test results.

EP10-A **Preliminary Evaluation of Quantitative Clinical Laboratory Methods; Approved Guideline (1998).** This guideline addresses experimental design and data analysis for preliminary evaluation of the performance of an analytical method or device.

EP15-P **User Demonstration of Performance for Precision and Accuracy; Proposed Guideline (1998).** This guideline demonstrates method precision and accuracy for laboratory analyte determinations, utilizing a protocol designed to be completed within five or fewer working days.

M29-A **Protection of Laboratory Workers from Instrument Biohazards and Infectious Disease Transmitted by Blood, Body Fluids, and Tissue; Approved Guideline (1997).** A consolidation of M29-T2 and I17-P, this document provides guidance on the risk of transmission of hepatitis viruses and human immunodeficiency viruses in any laboratory setting; specific precautions for preventing the laboratory transmission of blood-borne infection from laboratory instruments and materials; and recommendations for the management of blood-borne exposure.

NRSCL8-A **Terminology and Definitions for Use in NCCLS Documents; Approved Standard (1998).** This document provides standard definitions for use in NCCLS standards and guidelines, and for submitting candidate reference methods and materials to the National Reference System for the Clinical Laboratory (NRSCL).

* Proposed- and tentative-level documents are being advanced through the NCCLS consensus process; therefore, readers should refer to the most recent editions.

NCCLS ▾ 940 West Valley Road ▾ Suite 1400 ▾ Wayne, PA 19087 ▾ USA ▾ PHONE 610.688.0100
FAX 610.688.0700 ▾ E-MAIL: exoffice@nccls.org ▾ WEBSITE: www.nccls.org ▾ ISBN 1-56238-407-4